I. Significance and Relevance to SRP Mandate and Superfund

Per- and polyfluoroalkyl substances (PFAS) in drinking water is an emerging public health issue; recently it was estimated that 6 million Americans are using drinking water that exceeds the USEPA health advisory level for PFOA and PFOS [ADDIN EN.CITE <EndNote><Cite><Author>Erbentraut</Author><Year>2017</Year><RecNum>13</RecNum><DisplayText>[13]</DisplayText><record><rec-number>13</rec-number><foreign-keys><key app="EN" db-id="spp2ftdvxpr2t6ez5zrvr9wn25r5vs5ss0fw" timestamp="1501539045">13</key></foreign-keys><ref-type name="Web Page">12</ref-type><contributors><authors><author>Erbentraut, J</author></authors></contributors><titles><title>Report: 15 Million Americans' Drinking Water Tainted By Toxic Chemical</title></titles><volume>2017</volume><number>July 31</number><dates><year>2017</year><pub-dates><date>06/08/2017</date></pub-dates></dates><publisher>Huffington Post</publisher><urls><related-urls><url>http://www.huffingtonpost.com/entry/nonstick-chemicals-water-contamination_us_59398625e4b0061054809e64</url></related-urls></urls></record></Cite></EndNote>]. PFAS are persistent in the environment and soluble in water. PFAS are used in many industrial processes and are found in a wide range of consumer products. PFAS have been produced since the late 1940s. Due to the large number of fluorine atoms, these chemicals are very non-reactive which make them excellent for non-stick materials and firefighting foams, but create environmental hazards due to their environmental persistence. This chemical class includes over 3000 chemicals; PFOS and PFOA phase out began in the early 2000s, while other less environmentally persistent are more commonly used now {Wang, 2017 #5}. The older PFAS chemicals such as PFOA and PFOS are regarded as "legacy" chemicals while there are many newly identified chemicals such as GenX and Nafion byproduct 2. There is a paucity of public health information available for the newly identified chemicals. As a result of their widespread use, PFAS have been detected in drinking water, wildlife, and humans across the globe, and they are classified as contaminants of emerging concern [ADDIN EN.CITE ADDIN EN.CITE.DATA].

PFAS are important surface water contaminants in North Carolina. In 2013-15, 80% of NC drinking water samples that contained legacy PFAS were from surface water {, #10}. The Cape Fear River Basin, the largest watershed in NC and the drinking water source for 1.5 million people, is heavily impacted by PFAS [Figure 1]. There is high variability in specific PFAS chemicals and concentrations across the Basin (Nakayama et al., 2007) because there are multiple source inputs over the ~9,000 square miles the basin covers. Besides supplying drinking water, the Basin supports many industrial water users around the cities of Greensboro, High Point, Burlington, Chapel Hill, and Durham in the upper part, and Fayetteville and Wilmington in the middle and lower parts. Water quality in the upper part of the Basin is characterized by elevated levels of legacy PFAS (e.g., PFOA and PFOS). Legacy PFAS are frequently detected in the Haw River, the headwaters for the Cape Fear River, and the drinking water source for ~4,000 people in Pittsboro, NC. Samples from the Haw River often exceed EPA's health advisory level of 70 ng/L for PFOA and PFOS. In 2018, elevated levels (> 40 ng/L) of the legacy PFAS (PFBA, PFPeA, PFHxA, PFHpA, PFOA, and PFOS) were detected in the Haw River (Unpublished data). The sources of legacy PFAS are diverse; these include application of biosolids on land between Burlington and Chapel Hill and elevated PFOS from firefighting foams at Greensboro's airport. There is little evidence of legacy PFAS in the Cape Fear River north of Fayetteville, NC (Sun et al., 2016) suggesting that there are no significant PFAS sources between Pittsboro and Fayetteville. After Fayetteville, the PFAS signature of the Cape Fear changes significantly. Discharge from a fluorochemical manufacturing plant in Fayetteville, NC, introduces several novel PFAS, besides the legacy PFAS, to the river (Strynar et al., 2015, Sun et al., 2016). These novel PFAS are ether acids developed as alternatives for legacy PFAS (e.g., GenX replaced PFOA), or were byproducts of the plant's vinyl ether production line (e.g., Nafion byproducts). These PFAS were also found 90 miles downstream of the plant, at the drinking water intakes for the city of Wilmington and Brunswick County (Sun et al., 2016). USEPA data from 2018 underlines how much the plant's discharge impacts the Cape Fear. The total ion intensity, a measure of all chemicals, in water downstream of the plant was greater than 90% chemicals with a negative

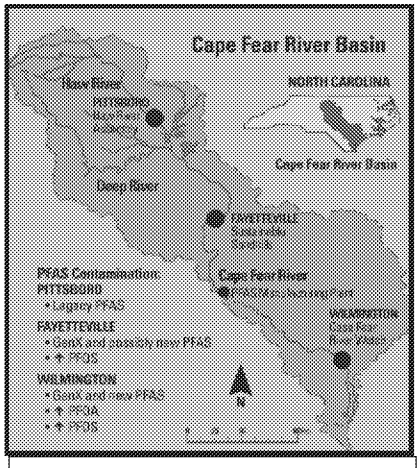

Figure 1: Cape Fear River Basin and PFAS Contamination

mass defect, an identifying feature of a PFAS. Twelve PFAS were found upstream, ten of which were legacy PFAS, but ~200 PFAS were found downstream, including 22 PFAS not described elsewhere (Unpublished). *One major challenge of PFAS is that they are not removed by standard water treatment, so PFAS levels in finished drinking water are the same as in the source water (Sun)*. Even though the PFAS manufacturing plant stopped discharging wastewater from its vinyl ether production line in mid-June 2017, GenX and other novel PFAS are still detected in drinking water at low levels in November 2017 and May 2018 (unpublished data).

PFAS are also important groundwater contaminants in NC. Groundwater serves as the primary drinking water source for the 1000s of residents living around the chemical plant in Fayetteville. Groundwater sampling in this region started in July 2017 and is ongoing. Current data suggest that well water is contaminated more than 5 miles from the plant. These wells appear to be contaminated as a result of airborne transport, as some wells are upstream of the facility. Efforts are ongoing to determine the extent of GenX contamination of groundwater around the plant; limited information is available for other PFAS. To address this concern, NC DEQ analyzed water from 17 wells adjacent to the Chemours plant for 30 additional PFAS. These wells had detected levels of both the legacy chemicals of PFOA and PFOS as well as some of the newly identified PFAS compounds including PFMOAA, Nafion byproduct 1 and 2. Groundwater can also serve as a non-point source of GenX and other new PFAS for the Cape Fear River. *For most of the well owners, there are no data on the vast majority of PFAS found in their home drinking water.*

While researchers had been identifying a whole suite of new PFAS in the lower Cape Fear since 2012 (Strynar et al., 2015; Sun), the residents and consumers of Cape Fear water were unaware of these findings. In June 2017, residents of Wilmington, NC, woke to the news that they had been drinking GenX and other PFAS potentially since 1980. In 2017, the levels of GenX in finished drinking water were approximately 700 ng/L; the state Department of Environmental Quality (NCDEQ) set a health goal of 140 ng/L in July 2017. Community engagement led to quick action by NCDEQ to require the chemical company to stop discharging GenX to the River, however, no information was available to the community regarding exposure and potential health effects of GenX and other PFAS. To meet this need, we submitted a time sensitive grant to NIEHS in August 2017 to measure GenX and other PFAS in tap water, blood, and urine; identify predictors of exposure; and assess clinical outcomes related to lipid and thyroid function. The grant (ES029353: Hoppin, PI) was funded in November 2017 and two weeks later with assistance of our community partners, Cape Fear River Watch and the New Hanover County Health Department, we enrolled and collected samples from 310 individuals; 34 additional participants were enrolled and 44 initial participants were resampled in May 2018. Tap water samples were analyzed for 17 different PFAS [Table 1]. These results were shared with individuals and the community in April 2018; GenX, PFOA, and PFOS were all detected, but at lower than the state and federal action levels. Additionally, we found five new chemicals in the drinking water sourced from the Cape Fear River (Nafion byproduct 2, PFMOAA, PFO2HxA, PFO3OA, and PFO4DA) for which we did not have analytical standards at that time. These water results informed the blood PFAS analysis and we were able to obtain chemical standards for the PFAS that we had identified in drinking water. Using high resolution mass spectrometry, we identified 12 PFAS in the blood of GenX Exposure Study participants. Six of these chemicals (Nafion byproduct 2, PFO4DA, PFO5DoDA, PFO3OA, NVHOS, and Hydro-EVE) have not been previously reported in human blood samples; we had analytical carboxylic acid of Nafion byproduct 2 (Table 2). GenX was not detectable in blood samples at 2 ng/mL. We did not detect these new chemicals in two other groups (blood samples from women in the Raleigh, Durham, Chapel Hill area in 2008-2009 and from residents of Dayton, OH, with known elevated PFOS exposure collected in 1992-2014). In addition to these new chemicals, levels of the legacy chemicals (PFOA, PFOS, PFHxS, PFNA, and PFDA) in our Wilmington sample exceeded the 2015-2016 NHANES levels {, 2018 #6}. As seen in Figure 2, the median concentration of all these chemicals is higher in the Wilmington sample.

Table 1 PFAS Analyzed in Tap Water and Blood Samples, Wilmington, NC, November 2017

Short Name	Chemical Name	CAS Number
GenX	Perfluoro-2-propoxypropanoic acid	13252-13-6
PFOA	Perfluorooctanoic acid	335-67-1
PFOS	Perfluorooctane sulfonic acid	1763-23-1
PFPeA	Perfluoropentanoic acid	2706-90-3
PFHxA	Perfluorohexanoic acid	307-24-4
PFHpA	Perfluoroheptanoic acid	375-85-9
PFBA	Perfluorobutanoic acid	375-22-4
PFNA	Perfluorononanoic acid	375-95-1
PFDA	Perfluorodecanoic acid	335-76-2
6:2 FTS	6:2 fluorotelomer sulfonate	27619-97-2
PFBS	Perfluorobutane sulfonic acid	375-73-5
PFHxS	Perfluorohexane sulfonic acid	355-46-4
Chemicals without analytic standards		
PFMOAA	Perfluoro-2-methoxyacetic acid	674-13-5
Nafion byproduct 2	Ethanesulfonic acid, 2-[1-[difluoro(1,2,2,2-tetrafluoroethoxy)methyl]-1,2,2,2-tetrafluoroethoxy]-1,1,2,2-tetrafluoro-	749836-20-2
PFO2HxA	Perfluoro(3,5-dioxahexanoic) acid	39492-88-1
PFO3OA	Perfluoro(3,5,7-trioxaoctanoic) acid	39492-89-2
PFO4DA	Perfluoro(3,5,7,9-tetraoxadecanoic) acid	39492-90-5
Additional Chemicals detected in Blood		
PFO5DoDA	Perfluoro-3,5,7,9,11-pentaoxadodecanoic acid	39492-91-6
NVHOS	1,1,2,2-tetrafluoro-2-(1,2,2,2-tetrafluoro-ethoxy)ethane sulfonate	801209-99-4
Hydro-EVE	2,2,3,3-tetrafluoro-3-((1,1,1,2,3,3-hexafluoro-3-(1,2,2,2-tetrafluoroethoxy)propan-2-yl)oxy)propanoic acid	773804-62-9

Table 2: Six newly-identified PFAS in the blood of GenX Exposure study participants (N=388)

		Percentiles (ng/mL)			
Compound	% > 0.1 ng/mL	25th	50th	75th	95th
Nafion byproduct 2	100	1.5	2.7	4.6	8.1
PFO4DA	98	0.8	2.2	5.3	12.4
PFO5DoDA	87	0.2	0.3	0.5	1.0

PFO3OA	31	ND	0.1	0.1	0.5
NVHOS	14	ND	ND	0.1	0.3
Hydro-EVE*	76	--	--	--	--
*No analytical standard for Hydro-Eve was available.					

Of particular concern is the elevated levels of PFOA; more than half of our standards for all chemicals except Hydro-EVE, a population exceeds the 95thpercentile for PFOA (the chemical that GenX replaced). Levels of PFOS (a chemical related to firefighting foam at airports and air force bases) are twice the median for the US population and comparable to those measured in Pease, New Hampshire, a recognized hotspot of PFOS exposure {Daly, 2018 #7}. Biomonitoring by NC DHHS has also been conducted among 30 Fayetteville residents whose wells exceed the state's health goal of 140 ng/L {, #9}. GenX was not detected in blood or urine of these individuals. The analytical method did not include the four new chemicals we detected {Kato, 2018 #8}; however, it did include all the legacy chemicals. PFOS, but not PFOA, were regarded as higher than the national data. *Taken together, these results suggest that: 1) residents of the lower Cape Fear Basin are exposed to newly identified PFAS however no data are available on these chemicals except GenX for Fayetteville residents; 2) the elevated levels of PFOS in both Fayetteville and Wilmington populations may have a potential upstream source; and 3) the elevated PFOA levels among Wilmington residents may be associated with upstream river contamination which is not seen in the private well community in Fayetteville.*

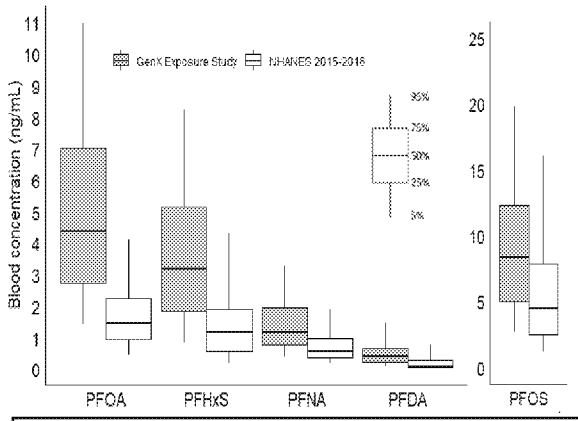

Figure 2: Legacy PFAS concentrations (ng/mL) in Wilmington, NC, 2017 and NHANES 2015-2016

A major challenge related to PFAS exposure is there is so little known about the biological half-lives of these newly identified chemicals in humans. Others have investigated the half-lives of PFOA, PFOS, and PHFxS in communities exposed via drinking water (Li, Brede, Bartell, Worley). These studies ranged in size from 65 (Brede) to 200 (Bartell) people with 2 to 7 samples collected per individual; two studies included children (Li, Brede). Three studies included all three PFAS, but evaluated the half-lives individually for each chemical and did not include the total concentration of PFAS in their calculations (Li, Brede, Worley). The data from these studies were consistent with first-order elimination. In a community based study of 106 individuals aged 4-84 years with seven measures of exposure collected over a 2 year period, Li et al estimated the half-lives to be 5.3 years for PFHxS, 3.4 years for PFOS, and 2.7 years for PFOA; with more rapid elimination of PFHxS and PFOS by women (Li). The authors (Li) concluded that "*further research to understand the determinants of elimination is needed … to guide risk assessment and regulatory measures*….' To do this larger sample sizes of diverse populations are necessary.

Of particular concern are differences associated with the serum half-lives of PFAS among experimental animal models and exposed humans. Differences by species as well as sex make it challenging to extrapolate and predict the half-life in humans who have been exposed to GenX and other PFAS in their drinking water for decades. For PFOA, the serum half-life varies widely by species, ~2 hours in female Sprague-Dawley rats, 2 weeks in male and female mice, and up to 5 years in humans. For GenX, the serum half-life is similarly variable, with the half-life longer in males than females [ADDIN EN.CITE ADDIN EN.CITE.DATA]; monkeys had the longest half-life (~64-80 hours) followed by rats (~23-90 hours), then mice (~24-37 hours). Our findings of no detectable GenX five months after the source was turned off, is consistent with a short half life. While we could not evaluate the half-life of GenX, we were able to assess changes in PFAS concentration over our six-month time period. Levels of all the newly identified PFAS dropped in six months; the median change in this period ranged from 27% for PFO5DoDA to 75% for Hydro-EVE [Figure 3 unpublished data], with no appreciable change in the concentration of legacy PFAS (<13%).

PFAS are associated with human health effects in environmentally exposed populations. Much of what is known about human health effects comes from the C8 Health Project, a community-based study of 69,030 individuals from six PFOA contaminated water districts as well as private well owners in West Virginia and Ohio (Frisbee). The study enrolled individuals from 2005-2006 and focused on exposures occurring from 1950 to 2004. PFOA exposure was assessed through both biological monitoring for current exposure and modeling for historic exposure estimation (REF). The C8 Science Panel considered high cholesterol, ulcerative colitis, thyroid disruption, testicular and kidney cancer, and pregnancy induced hypertension as probably associated with PFOA (C8 website). In children, health outcomes associated with PFAS include dyslipidemia, adverse immune response, asthma, renal function, and age at menarche, and thyroid function (Rappazzo, Ballesteros). In adults, PFOS has been associated with total cholesterol, glucose metabolism, body mass index, and thyroid function (Saikat; Ballesteros). Children exposed in utero have an increased risk of obesity (Braun).

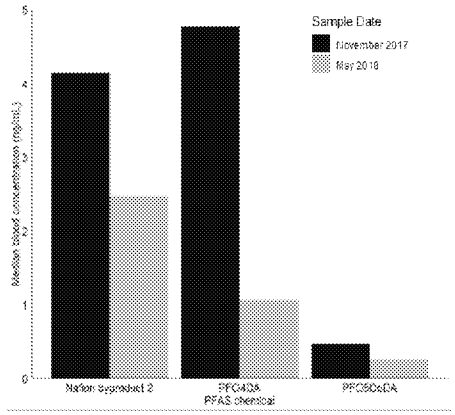

Figure 3: Changes in median PFAS levels from November 2017 to May 2018, n =44

We consider thyroid outcomes the sentinel outcome of PFAS because PFAS may directly impact thyroid function via alteration in thyroid hormones or by secondary effects on cholesterol, weight, and heart rate. The thyroid is key for healthy living at all ages and therefore, it is a relevant outcome for our diverse population.

PFAS are associated with thyroid outcomes in both laboratory animals and humans. The thyroid is an endocrine organ that is particularly susceptible to endocrine disrupting chemicals (Gore). Thyroid hormones are crucial to the growth, development, and metabolic function of organ systems in the body. Both overproduction (hyperthyroidism) and underproduction (hypothyroidism) of thyroid hormones can adversely impact health. Thyroid hormone production is a complex interplay among the hypothalamus of the brain which secretes thyrotropin releasing hormone, the anterior pituitary gland which secretes thyroid stimulating hormone (TSH), and the thyroid gland itself which secretes the thyroid hormones (TH) triiodothyronine (T3) and thyroxine (T4) (Dong and Greenspan, 2015). Complex feedback loops balance the production of these hormones as well as dietary iodide, which is necessary for the biosynthesis of thyroid hormones, and efficacy of a variety of enzymes involved in TH biosynthesis and peripheral conversion (Dong and Greenspan, 2015). TH is mostly bound to plasma proteins (thyroxine-binding globulin, transthyretin, and albumin) while in blood circulation; only 0.04% of T4 and 0.4% of T3 are free, but this free component is the biologically active component (Cooper and Ladenson, 2011). Dysregulation of thyroid hormones can adversely impact individuals throughout the lifecourse, with adverse impacts observed from infants to the elderly. *That is, the impacts of PFAS on thyroid can adversely affect the health of an entire exposed community, not just to a subset of the population.*

PFOS has been associated with alteration in thyroid in animal models; information on other PFAS are limited. PFOS exposure during adulthood has been associated with suppression of TH in monkeys (Seacat et al., 2002) and in rats and mice (Thibodeaux et al., 2003). Developmental exposure to PFOS leads to T4 suppression in rats (Lau et al., 2003). Other rodent studies have reported decreases in serum T4 (Curran et al., 2008; Yu et al., 2009a,b) without changes in T3 and/or TSH, which suggests possible interference with biosynthesis or peripheral conversion enzyme. Rat pups exposed *in utero* to PFOS showed a statistically significant decrease in free T4 and an increase in TSH—which is indicative of hypothyroidism (Luebker et al., 2005, 3M_MN03095624).

In epidemiologic studies of populations from infants to the elderly exposed to PFAS, clinical measures of thyroid function (TSH, free and total T3, free and total T4) have been evaluated. Most of these studies have focused on PFOA or PFOS and have evaluated the associations separately for each chemical. In a recent meta analysis of 12 papers focusing on adult outcomes, increasing PFOS was associated with increased free T4 and TSH and decreasing total T3 and total T3, consistent with hypothyroidism (Kim et al PLOS One 2018). The associations were stronger in the middle range of PFOS (8-16 ng/ml), *similar to the levels measured in Wilmington, NC.* The association of PFAS (PFOA, PFHxS, PFOS, and PFNA) and thyroid outcomes has been

evaluated in four papers using data from NHANES (Jain, Wen, Melzer, Webster). Prevalent self-reported thyroid disease was associated with increased PFOA and PFOS in both men and women in an analysis using NHANES 1999-2006 data [Melzer]. The other papers included thyroid hormones as the outcome and all included the NHANES 2007-8 data set (Jain, Webster, Wen); Wen also included the 2009-10 NHANES data (wen). Collectively these papers showed associations for PFOA and PFHxS and thyroid outcomes. The associations were strongest in people who were iodine deficient and thyroid peroxidase antibodies, suggesting that thyroid stress could make individuals more sensitive to PFAS. While all these papers had measures of multiple PFAS, none of them mutually adjusted for other PFAS. In utero PFAS exposure has been shown to impact thyroid hormones in offspring [Kato, others]. Perturbations on the thyroid axis can lead to altered metabolic status which may manifest in dyslipidemia, weight changes, and carbohydrate metabolism. These secondary outcomes of altered thyroid function have been seen in community exposed populations with the strongest association for cholesterol and PFOA [Rappazzo]. Interestingly, higher levels of PFOS and PFNA were associated with greater weight regain among 621 adults participating in a weight loss program, potentially due to slower return to normal resting metabolic rate levels (Liu et al 2018). *Many of the studies to date have been cross-sectional, prospective evaluation of the impact of PFAS on thyroid related health outcomes is critical.*

This project is relevant to the Superfund program and its mandates. PFAS have been detected at a number of Superfund sites, and this number is increasing as communities advocate for testing of PFAS in landfill leachate from Superfund sites. The University of Rhode Island's Superfund Center, STEEP, is focusing on legacy PFAS. Our inclusion of both newly identified PFAS and legacy PFAS complements the work by STEEP. This community-engaged human exposure project is consistent with two of the four SRP mandates: (a) advanced techniques for the detection, assessment, and evaluation of the effects on human health of hazardous substances, and (b) methods to assess the risks to human health presented by hazardous substances.

II. Investigators

This project brings together an interdisciplinary group of investigators with expertise in community engagement, PFAS chemistry, toxicokinetic modeling, epidemiologic modeling, science communication, environmental impacts on thyroid function, medicine, and public health. The investigator team brings together scientists from NC State, ECU, University of North Carolina at Chapel Hill, and University of Nebraska Medical College. The project is nested in the Cape Fear River basin and has formal partnerships with three community organizations (Haw River Assembly, Sustainable Sandhills, and Cape Fear River Watch).

Dr. Jane Hoppin will lead this project. She is an environmental epidemiologist, Associate Professor in Biological Sciences at NC State, and Deputy Director of the NC State Center for Human Health and the Environment (CHHE). She is the PI of the GenX Exposure Study (ES029353) and has extensive experience in environmental exposure assessment, epidemiology, reporting back results to communities, and leading multi-disciplinary studies. She has a history of collaboration with all the investigators and many of the Partners. She will oversee all aspects of this project. Co-Investigators from the GenX Exposure Study will contribute to this project. From NC State, these are **Dr. Detlef Knappe** and **Ms. Katy May**; from ECU, these are **Dr. David Collier** and **Dr. Suzanne Lea**; and from USEPA, these are **Dr. Andrew Lindstrom** and **Dr. Mark Strynar**. The investigator team for the GenX Exposure Study will be expanded to include experts in toxicokinetic modeling (**Dr. David Reif**), epidemiologic modeling and analysis of mixtures (**Dr. David Richardson**), and the impact of chemicals on thyroid function (**Dr. Whitney Goldner**).

Dr. Knappe is Professor in Civil and Environmental Engineering, Deputy Director of the SRP Center, and leader of the NC PFAS Testing Network. He has decades of experience related to PFAS in water and sharing results with communities, regulators, and utilities. He will contribute to interpretation of PFAS findings, exposure reconstruction, and report back efforts. **Ms. May** is the Community Engagement Core (CEC) director for CHHE and co-Deputy Director of the CEC for the Superfund Center. For the GenX Exposure Study, she has contributed to all community report back efforts including website development, Community Science Advisory Board engagement, preparation of materials for report back, and advising scientists on best practices for community presentations. **Dr. Collier** is a practicing pediatrician, co-leader of the CHHE Integrated Health Sciences Facility Core, and an expert in childhood obesity. Dr. Collier will oversee biological sample collection and processing, will contribute to participant report back, and will respond to participants' concerns about

medical issues. **Dr. Lea** is an environmental epidemiologist, Associate Professor of Epidemiology at ECU, and former president of the NC Public Health Association. She will contribute to all aspects of the study in particular to questionnaire data collection, report back of results, and liasing with public health officials in the Cape Fear River Basin. **Drs. Lindstrom** and **Strynar** are chemists at USEPA and leaders in understanding PFAS contamination in the Cape Fear River Basin; the methods used for PFAS in this study and the GenX Exposure Study were developed in their laboratory. They will provide expertise on PFAS chemistry, report back, and provide input on all aspects of the study. **Dr. Reif** is a bioinformatics specialist, Associate Professor of Biological Sciences, and Director of the SRP DMAC. He has extensive expertise in toxicokinetic modeling and extrapolation of human dosimetry to animal models including PFOA and PFOS. He will contribute to the development of toxicokinetic models and exposure reconstruction activities. **Dr. Richardson** is an occupational epidemiologist, Associate Professor of Epidemiology at UNC-CH, and an expert in strategies to address chemical mixtures in epidemiologic studies. As a co-investigator on this project, he will provide expertise on modeling of mixtures and interpretation of the toxicokinetic results and exposure reconstruction efforts. **Dr. Goldner** is an endocrinologist at University of Nebraska Medical College. Her research focuses on the endocrine disrupting effects of environmental chemicals on the thyroid axis. She will contribute to the epidemiologic analyses of thyroid and will provide medical guidance on these topics. Our Community Partners (**Cape Fear River Watch, Haw River Assembly and Sustainable Sandhills**) are integral to the success of the project. They are active community-based organizations focusing on water and PFAS issues and will provide key reality checks on exposures, outcomes, and community concerns. This diverse group of experts and community partners provide the necessary experience to achieve this complex community-engaged project to understand PFAS exposure, toxicokinetics, and thyroid outcomes in the Cape Fear River basin.

III. Innovation

This prospective epidemiology project is highly innovative as it is the only study to address exposure and health effects associated with both newly identified and legacy PFAS. With repeated measures of PFAS from 1200 individuals from three different communities, our project will allow us to make inference about many PFAS concurrently. A recent review article on the human health effects of PFAS highlighted the need for ***"Large, prospective studies with repeated exposure assessment in independent populations are needed to confirm some suggestive associations with certain endpoints."***[Chang, et al. 2016]. We will include residents along the Cape Fear River who are exposed to PFAS in drinking water from either municipal water from the river or well water contaminated as a result of airborne transport, allowing us to look at different exposure profiles and potentially identify sources of PFOS and PFOA seen in Wilmington residents. The GenX Exposure Study has measured 12 different PFAS in the blood of Wilmington residents, six of which are newly identified. These newly identified chemicals are unique to chemical production at Chemours in Fayetteville, so there are few exposed populations globally. Because PFAS exposure is a problem globally, evaluating predictors of exposure, toxicokinetics, and health effects to 12 or more PFAS will allow us to create omnibus predictive models for PFAS as a whole which can inform us about other new PFAS as they are identified. We will use nontargeted analysis so that we can identify new PFAS in blood and urine.

We are studying a diverse population that includes young children and the elderly. This will allow us to evaluate the impact of these chemicals over the life course as well as assess toxicokinetic differences by age. Many studies include either children or adults, but by including all residents of a population, we will be better able to answer questions that address the health of the whole community, not just a subset. We have a track record of enrolling minority participants and engaged partners eager to help facilitate future recruitment as well as report back of findings in a culturally appropriate manner for the community (*See LOS from Deborah Maxwell, Amanda Boomershine).*

Finally, our community engaged project ensures that the Community gets the answers to their questions in a timely fashion. Our commitment to regular report back and data sharing will allow the community to become more engaged in science and be better advocates for their environmental health.

IV. Approach

To evaluate the impact of PFAS exposure along the Cape Fear River, we propose four specific aims designed specifically to address community concerns. These aims focus on community engagement to ensure that the impacted communities have their questions answered in a timely and appropriate manner (**Aim 1**), expansion of the GenX exposure study to more communities along the Cape Fear River, analysis biological samples for

PFAS in an untargeted manner (**Aim 2**), evaluation of toxicokinetics of this complex mixture of PFAS in a diverse population (**Aim 3**), and evaluating the impact of PFAS exposure on thyroid function and related health outcomes (**Aim 4**). This project will address two Superfund program mandates: 1) detection, assessment, and evaluate of human health effects of hazardous substances and 2) assessment of the risks to human health posed by hazardous substances.

Community: This project is grounded in communities living along the Cape Fear River and designed to answer community questions about PFAS exposure and human health. Specific PFAS chemical exposures may differ among these communities, but all communities have concerns about PFAS in their drinking water either from surface water or groundwater. To ensure active community engagement in our study, we propose **Aim 1: Partner with local community groups to address community concerns and share results with affected communities in a timely and appropriate manner.** This study, like its predecessor the GenX Exposure Study, is focused on providing answers to community questions and concerns about PFAS. To this end, we have partnered formally with three community groups (Haw River Assembly, Sustainable Sandhills, and Cape Fear River Watch) to connect with communities through hosting community meetings, advertising the study to participants and the community, identifying potential candidates for the Community Science Advisory Board, and sharing study findings with their broader audiences. These three community groups are formal partners with the SRP Community Engagement Core (CEC) and have provided letters of support for this project. There are many groups concerned about PFAS in North Carolina and our study plans to ensure that not only local communities, but also public health officials and regulators are updated in a timely fashion regarding study findings (see LOS from Zack Moore).

We will build on our experience with the GenX Exposure Study and follow Community Engagement Best Practices. The GenX exposure study had two formal partners (Cape Fear River Watch and New Hanover County Health Department) and many informal partners as the project progressed (Cape Fear Public Utility Authority, New Hanover County NAACP, Spanish professor and her students at UNC-Wilmington, NC Department of Health and Human Services, Port City Community Church Spanish Lay Ministry). We anticipate that our proposed project will use a similar snowball method for recruiting and engaging people from diverse backgrounds [KM REF]. *Our project has defined roles for researchers and partners.* The research questions directly respond to input from and questions raised by Community Partners and other stakeholders. These questions include: What chemicals are in me? (**Aim 2**), How long have they been there? (**Aim 3**), What are the human health effects (**Aim 4**)? Community partners will engage local residents in the study, drive research questions, and provide input on report back, dissemination, and next steps, which will be complemented by the researchers' epidemiology, toxicology, and analytic expertise. *We will create opportunities throughout the course of the study to get input and advice from community members via Community Science Advisory Panels, reporting findings to individuals and communities, maintaining an updated study website, and a commitment to timely responses to participant emails and phone calls.* Additionally, our study will continue to inform and engage the public health community in a timely and meaningful way; for the GenX Exposure Study, we held a webinar specifically for local, state, and federal public health agencies to provide current study updates before they became public. This engagement process is iterative and designed to help generate information for the community while helping the community learn more about research versus clinical medicine. This engagement process complements the goals of the CEC to enhance environmental health literacy among communities in the Cape Fear River Basin by supporting improved understanding of individual and community-level exposures to PFAS.

We will work with community throughout the study. Key to this will be communication, both with our partners and the community as a whole. At the outset of the study, we will meet with all partners and identify additional community groups with potential interest in the study. By identifying additional community groups that cater to minority and under-represented populations, both our study recruitment and our study findings will reach these populations. Our community meetings will include informal meetings to assess interest, as well as intentional inclusion in investigator meetings to ensure that research is responsive to community questions. We will use press releases, the study website, the Center's social media platforms, as well as those of our partners to inform people about the study and its findings. Our partners will reach out within the community to ensure that the study represents the diversity of the community. *We will assemble three Community Science Advisory Panels (one for each region of the study) to provide input on the study, particularly report back of findings to the community*. The advisory panels will include community partners, local public health officials, local

healthcare practitioners, and concerned citizens who represent the diversity of the population. We anticipate one to four meetings annually held within the impacted communities, with more meetings during times when we are preparing to share information. Individuals may rotate on and off the Community Science Advisory Panel as their time and interest allows. The Community Science Advisory Panel for the GenX Exposure study provided key insight into the changing concerns of the community (e.g., how did we shift from GenX to PFAS more broadly?) and critical guidance for dissemination (e.g., making a video to help participants understand their report back letter). For study participants, we will provide them with their individual results for all PFAS measured in a timely fashion; we have used strip plots in the past based on methods applied in DERBI [KM REF]. For the larger community, we will host meetings and attend other local events to update the community on the findings of the study. We will host at least one community meeting per year in each community; venues that will be appropriate and accessible will be identified by community partners. We anticipate that there will be followup requests to participate in other community panels, local radio shows, or to address specific community groups. While most study investigators have experience communicating about PFAS to the public (Hoppin, Knappe, May), all study staff will work with the CEC to learn and build on best practices for discussing complex scientific information. Our community partners are also available to advise the study on local resources for individuals with health concerns identified through study participation (e.g., actionable high cholesterol). Through our formal community engagement process, we feel that this PFAS Exposure Study will address both important scientific questions, but also, and more importantly, community questions about PFAS and research.

Study Population: Working with established community partners, we plan to **characterize PFAS exposure among people living along the Cape Fear River (Aim 2).** We will recruit and follow three populations along the Cape Fear River from source to sea: Pittsboro, Fayetteville, and Wilmington. This will allow us to evaluate how PFAS exposure changes throughout the river basin. We have established one cohort in Wilmington of 344 people aged 6 to 86 years and will create another cohort of 200 people from the private well community around the chemical plant in Fayetteville, NC, in January 2019. Here, we propose to recruit individuals from Pittsboro, expand the Wilmington cohort, and continue to follow all three communities. We will collect blood and urine samples in Years 1 and 3 to evaluate PFAS exposures. This expanded cohort of 1200 individuals with multiple measures of exposure over time will provide a rich dataset to understand PFAS.

Recruitment and sampling strategy: We plan to recruit and follow a total of 1200 individuals in the Cape Fear Region (700 in Wilmington, 200 in Fayetteville, and 300 in Pittsboro). We anticipate that ~20% of our population will be children ages 6-17 years at enrollment; similar to our Wilmington sample. This study will include existing GenX Exposure Study participants in Wilmington; the January 2019 participants from the private well community around the Chemours Facility in Fayetteville, NC; and new participants recruited in the Wilmington region (Brunswick and Pender Counties) and Pittsboro, NC. Consistent with our initial recruitment strategy, we will recruit volunteers from these communities who are 6 years or older, have lived in the Community since July 2016, be willing to participate in a longitudinal study, and not pregnant, HIV or Hepatitis C positive at time of enrollment. Up to four residents per household will be eligible. Participants must be available to attend the sampling event in their region and be willing to provide blood and urine samples and complete a questionnaire. In Wilmington and Pittsboro, we will announce a call for volunteers for the study, approximately one month prior to the scheduled sample collection event. Individuals will be instructed to call our recruitment number for eligibility screening and sample collection scheduling. In order to ensure a diverse sample, we will create selection priorities for non-white minorities and families with children.

Table 3: Characteristics of GenX Participants (n = 344)

	Adults (n=289)	Children (n=55)
Characteristic	%	%
Gender		
Females	67	38
Males	33	60
Transgender		2
Race/Ethnicity		
White	78	69
Black	11	5
Hispanic	7	22
Other	4	4
Age		
6-10		33
11-14		47
15-17		20
18-29	5	
30-49	34	
50-69	45	
70+	15	
Years resident in Cape Fear Region		
1-5	12	11
6-10	15	36
11-19	27	53
20+	45	--

In the Wilmington area, we will expand our reach into Brunswick and Pender Counties to include a more diverse population of consumers of municipal water sourced from the lower Cape Fear River. We will target our recruitment in minority communities, including the community of Navassa, NC, which has a history of environmental justice concerns (see LOS from Veronica Carter). In Pittsboro, we will target municipal water users and recruit Hispanic participants

using a similar strategy to what was used in Wilmington to achieve an ~7% Hispanic sample. Activities include flyers in Spanish, informational events at local Mexican grocery stores, coordinating with informal networks in the Hispanic community, and ensuring translators for all research activities including websites and report back. No additional participants will be recruited among the private well community in Fayetteville.

In Wilmington, NC, we recruited 344 volunteers ages 6 and older who had lived in houses served by the Cape Fear Public Utility Authority (CFPUA) at least one year since July 2016 (~1 year before the GenX concern was identified). Our initial recruitment took place in November 2017, with a second recruitment in May 2018 designed to increase the participation of African Americans. Our sample represents 230 households and includes 289 adults and 55 children ages 6-17 years. The sample represents the diversity of Wilmington with 11% Black, 7% Hispanic, 4% Other Race, and 78% White residents [Table 3]. A majority of participants have lived in Wilmington for 11 or more years. While this is a volunteer cohort of concerned citizens, 49% of adults and 75% of children reported having very good or excellent health. This suggests that while people are concerned about their health, we have not created a cohort of individuals who are sicker than the general population.

In Fayetteville, NC, we received a supplement to our time sensitive R21 to extend the GenX Exposure study to the private well community around Chemours. Under this supplement, we will recruit 200 people whose wells have been sampled for GenX by NCDEQ or Chemours. Using a list provided by NCDEQ, we will create a stratified random sample where we select 100 people whose wells exceeded 140 ng/L and 100 people whose wells had lower levels. Because this community uses private wells, we need to sample from a list of people with known GenX concentration data unlike relying on utility data for Pittsboro and Wilmington. Other than this difference, eligibility criteria are the same. We had planned this work in Fall 2018, but Fayetteville was adversely impacted by Hurricane Florence, so we delayed field work to early 2019. We will continue to follow these individuals as part of this proposal.

Data Collection: We plan to collect biological and clinical data in Years 1 and 3 and additional questionnaire information in Year 5. We will be able to combine the data from the GenX Exposure Study in Wilmington and Fayetteville with this new data collection. We will collect biological samples, questionnaires, clinical measures of height, weight, and heart rate from all participants at community-based "pop-up" sampling events in Years 1 and 3. We will use a "pop-up" model of sampling events, so that all individuals are measured at the same point in time. This is critical because we are interested in how levels are changing over time. To encourage community participation, we will invite people to come to trusted locations in the community over multiple weekends. In the two sampling events conducted in Wilmington, one was at the New Hanover County Health Department and the other was at a local community center in conjunction with an NAACP-sponsored health fair. At these locations, people will be informed about the study, provide informed consent, donate a blood and urine sample, complete a study questionnaire, and have their height, weight, and heart rate measured. Blood samples will be collected by contracted phlebotomists from the local community; blood samples will be processed in an appropriate laboratory facility (e.g., County Health Department clinical laboratory) by study staff led by Dr. Collier. All blood and urine samples will be stored on ice during the sample collection weekend; afterward they will be transferred to -80 freezers at ECU and NC State prior to chemical and clinical analyses. Additional biological samples will be stored for future analyses.

Questionnaires: We will use questionnaires to collect residential history, water use information, demographic characteristics, medical history, current health status, and other potential PFAS exposures (e.g., carpeting, microwave popcorn, etc.). Questionnaires will collect detailed information on thyroid outcomes including diagnosed disease, medication use, and family history of thyroid disease. In Years 1 and 3, questionnaires will be administered in person at the time of blood collection to ensure complete and timely information; in Year 5 we will administer the questionnaire over the phone to update medical history and information on confounders and potential PFAS exposures. We will use questionnaires to create a retrospective PFAS exposure profile based on linking address history to municipal or private well information.

Participant Retention and Loss to Followup: Key to this project is the ability to follow people throughout the course of the study. In our community-engaged framework, we plan to remain in contact with participants throughout the course of the study with regular report back of study findings and activities. We will host community meetings to keep the whole community informed about the study. Through regular mailings, we will

be able to keep track of study participants and update their addresses as necessary. We anticipate some loss to followup due to moving out of the area; but given the enthusiasm in this region about understanding PFAS exposure and health effects, we anticipate that study dropout will be low. To date in the GenX exposure study, we have had one family move out of the area permanently and two families displaced as a result of Hurricane Florence. We will continue to follow people who remain in the area and, for those who leave the area but are still interested in participating, we will administer questionnaires over the phone to update health status.

PFAS measurement in biological samples: Serum samples will be quantified for both the newly identified and legacy PFAS in SRP Analytical Core (CAPTURE) using a parallel reaction monitoring approach on the Tribrid ID-X mass spectrometry platform. The method will initially focus on the PFAS already identified where stable-isotope labeled (SIL) internal standards are available or are being made in collaboration with Cambridge Isotope Laboratories (see CAPTURE). However, using the AcquireX data-dependent approach on the ID-X and the ability to use collisional cross section from the ion mobility TOF (time of flight) (see CAPTURE) will be able to identify new compounds of emerging concern as well. For these newly identified PFAS, CAPTURE will commission the synthesis of the SIL standards so that any new chemicals detected in blood can also be quantified. The data will provide quantitative estimates of PFAS exposure. Importantly, we do not know whether PFAS are detectable in urine as these biospecimens have not yet been analyzed from the GenX Exposure Study. However, we anticipate that some of the smaller chain PFAS such as PFMOAA which was found in the water samples from both November 2017 and May 2018, will be found in urine. If we do not detect PFAS in the urine samples from the GenX Exposure study, we will store the urine samples for future analyses of other chemicals of concern in the region (e.g., 1,4-dioxane).

Clinical Analyses: We will analyze all blood samples collected in Years 1 and 3 for markers of thyroid function and resulting impact on metabolic outcomes. We propose the following measures: 1) Markers of thyroid function (thyroid stimulating hormone (TSH), total T4, free T4, total T3, free T3, anti-thyroperoxidase (TPO) and thyroglobulin antibodies) to evaluate thyroid gland function; 2) lipid panel (total cholesterol, HDL cholesterol, triglycerides, LDL cholesterol, with Non-HDL cholesterol calculated) to assess changes in lipid levels; and 3) Markers of liver function (alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), bilirubin, total protein, and albumin) as part of a comprehensive metabolic panel to screen for liver damage. In urine, we will analyze iodine, creatinine, protein, and specific gravity. We will use the urine specific gravity results to adjust the PFAS in urine results for dilution. In Year 5, we will conduct additional clinical analyses on a subset of stored samples to address community concerns about PFAS exposures. These may include immune toxicology measures identified in BMR Project 2, markers of puberty in the children, or other topics identified by our Community Partners and Community Science Advisory Panels. Dr. Hoppin will work with these groups to ensure that a valid epidemiologic question will be answered.

Statistical Analyses: To address the three scientific aims of this proposal, we will use statistical approaches that address the complexity of the PFAS data. From the GenX Exposure Study and the newly collected samples from this project, we will have measured five calendar years with a minimum of two samples from every individual and up to 4 samples from 44 individuals; Table 4 details the available samples from each group during the course of the study. We will have measured at least 12 PFAS in the blood of all individuals and may identify more chemicals through our untargeted chemical analysis of serum and urine samples.

Table 4: Number of PFAS Samples by Region and Calendar Year

Cohort		2017	2018	2019	2020	2022
Wilmington	Group 1	310	44		300	300
	Group 2		34		30	30
	Group 3				370	370
Fayetteville				200	200	200
Pittsboro					300	300
Total		310	78	200	1200	1200

Aim 2: Characterize PFAS exposure among people living along the Cape Fear River. Using the results of PFAS in blood and urine, we plan to characterize exposures to individuals and communities along the Cape Fear River. We want to compare communities over time and space because we anticipate exposures changing during our study efforts. The most important predictors for PFOA from the C8 Study were drinking water level at home and work, employment at the chemical plant, gender, consumption of local vegetables, and age (Steenland). Through our inclusion of families, we will be able to compare people within the same household; the C8 Health Project evaluated the relationship between mothers and children for PFOA and PFOS levels and saw that children had higher concentrations than their mothers (Mondel). For each community, we will start

with a descriptive analysis of the distribution of measured values for each PFAS and trend within community over time. Trends in mean values, as well as quantiles, will be examined for each PFAS in each community. We will compare values to other population-based samples such as NHANES (REF) to help interpret the values for the community. Next, a hierarchical regression model will be developed to describe the joint distribution of PFAS chemicals, allowing for within subject effects as well as between subject factors. Joint modeling of the 12 PFAS chemicals and information for all individuals across communities will allow us to place a hierarchical structure on predictive factors that may affect patterns of PFAS (that result in correlation of the measured values as well as change over time due to differences in biological half-lives of the chemicals). The joint modeling will also allow us to evaluate the relative importance of factors to each PFAS evaluated. Factors to be examined include region, race, sex, age, years on water source, employment at the chemical plant, consumption of local fruits and vegetables, and sampling year. Correlation in measures within subject can be modeled by subject terms, while correlation between measures can be modeled flexibility, allowing a structure on explanatory variables implied by a hierarchical modeling approach. Models will be fitted using the SAS software package.

Toxicokinetics: **Aim 3: Evaluate the toxicokinetics of PFAS in this population.** One key question from the community is: how long have I been exposed? Using repeated blood and urine samples, we will follow the changes in PFAS over time and use this information to predict past exposures. For Wilmington and Fayetteville, we have collected samples at multiple points of time after the source was turned off; for Pittsboro, PFAS exposures are ongoing, but may change during the course of our investigation. We will assess whether toxicokinetics differ by age, gender, race, age, physical characteristics such as BMI, and presence of other PFAS. Using data on the 12 PFAS found in Wilmington as well as any new ones we identify in this project, we will be able to create a predictive model to provide insight on the toxicokinetics of future PFAS. With the diverse PFAS exposure history, multiple measurements over multiple years, and a diverse population with respect to age, race, and years of exposure, we plan to create an omnibus toxicokinetic model that can be used to make inference not only about the 12 chemicals measured here, but additional PFAS found in the US and around the world.

Building an Omnibus Toxicokinetic Model of PFAS: To do this, we propose a Bayesian cheminformatics approach to model retention/excretion for PFAS incorporating multiple compartments and utilizing the in vivo measurements of human plasma and urine levels. To optimize the form and parameterization, cross-validation methods will be used. We will recombine our sampled data with multiple streams of external data to add more global, omnibus context to our local model. Predictive factors will include both individual characteristics (e.g., age, gender, race, BMI) as well as chemical characteristics (e.g., carbon chain length). Optimizing the model for these factors will facilitate application of the omnibus model in other settings or for other PFAS exposure scenarios. For the historically used PFAS, there are other populations with repeated measures including the C8 study (Bartell), and others (Li, Olsen, Brede, Worley). To the extent possible, we will incorporate these data into our toxicokinetic modeling to extend the concentration range and to include different ratios of PFAS (i.e., groups with higher PFOA and lower PFOS). Additionally, we will mine the growing body of data deposited on public databases for PFAS. Our chemoinformatic approach will incorporate chemical characteristics (e.g., carbon chain length) with the biologic sampling data. The benefits of including external data are reduction of uncertainty (i.e., higher resolution credible intervals), assessment of the generalizability of our models beyond the collected compound set, and opportunities to link new data sources. A salient advantage of our Bayesian framework is that additional data instances (PFAS) and attributes (measured quantities) augment model-building strength, and we can use that strength to deal with missingness and other common issues that arise in geosampled data [REF Tilley 2017; REF To 2018]. These high-dimensional, external data have proven useful for integrating exposure data into biomedical, prioritization, and toxicokinetic applications [REF Grondin 2016; Gangwal 2012; REF Rotroff 2010].

Using Toxicokinetics to Model Historic PFAS levels: Leveraging the multiple geographic sites, biological samples, and repeated sampling times, we will create a local model that reconstructs a "most likely" scenario to predict past exposures (i.e. those that predate the sample collection). For potential historic water levels of PFAS, we will incorporate measured PFAS data from the Cape Fear River (Sun, Strynar, others), from the Fayetteville private well community collected as part of the NCDEQ investigation and through the NCPFAST network, and from the Cape Fear Public Utility Authority. For biological samples, in addition to the results from this study, we will include PFAS results from historic blood samples collected in this region from UNC

Lineberger's Health Registry/Cancer Survivorship Cohort. We plan to analyze blood samples from ~200 adults from this cohort who were living along the Cape Fear River collected between April 2010 and August 2016; these data will be available for this modeling effort. Because the proposed sample will grow in size throughout the first three years of the study, we will implement the model within a Bayesian framework that can be refined as additional data are added [REF Wilson 2014]. Priors for the initial model will cover a broad space estimated using data from the GenX Exposure Study data where results will be used to identify which parameter(s) covers the broadest uncertainty space. As data from all sampling sites arrive, we will implement a full Bayesian pharmacokinetic (PK) model that incorporates all data to predict past exposure, akin to the dosimetric anchoring approach we developed for PFAS in [REF Wambaugh 2013 Tox Sci]. Bartell developed a PFOA calculator for the C8 study participants (REF); *our proposed model will build on this model and provide inference about additional PFAS.* Our omnibus model will be calibrated against far-field models of exposure to estimate how well our measured set of compounds represents the full exposome of PFAS [REF Wambaugh 2013 Env Sci Tech; REF Pearce 2017]. As our models are refined and moved to dissemination in public resources, the scientific community can use our results as epidemiologically-based estimates of PFAS exposure, where only the priors (i.e. starting assumptions) have been calibrated with external data.

Health Outcomes: **Aim 4: Assess the impact of multiple PFAS on thyroid function over the lifecourse.** Alteration in thyroid hormones can have impact on human health across the lifespan. In our cohort ranging in age from 6 to 86, we will measure thyroid hormones, thyroid antibodies, and potential secondary effects of thyroid disruption including weight, cholesterol, and heart rate to be able to prospectively evaluate the association of PFAS in different age groups. We will also assess measures of thyroid stress including urinary iodine level and serum thyroid peroxidase (TPO) antibody levels (Webster). We will use of repeated measures as well as reconstruction of historic exposures to mitigate previously identified concerns about reversal causality from cross-sectional studies (Dhringa).

The modeling approach will involve a mixed model to allow for repeated measures of the outcome variable on a subject. We have multiple thyroid outcomes from serum (TSH, Free T4, Free T3, Total T4, Total T3, thyroid binding proteins including albumin), most measured on a continuous scale; and, these will be examined one-at-a-time. We will evaluate the secondary outcomes of lipids, weight change, and heart rate in a similar manner. For a given exposure variable of interest, we face the challenge that the outcome measure at time, t, denoted O(t) may be associated with the exposure of interest at t, denoted E(t), or previous measures of that value E(t-1), E(t-2). While long term lags are not feasible to examine in this study, we do know that our preliminary results suggest relative short biological half-lives for the newly identified PFAS suggestive of greater importance of exposures proximal in time to thyroid hormone level measures than those in the distant past. Potential confounders in these longitudinal (i.e., repeated measures) models include fixed covariates such as subject race and sex, as well as time-varying factors such as attained age, body mass index.

In addition, in our models we wish to examine the potential confounding or modifying effects of the other PFAS exposures that have been measured on each subject at each sampling time. Multiple PFAS pose analytical challenges given potential collinearity of some of these measures. Given the need to flexibly jointly model the effects of a series of PFAS concentrations measured per subject, with number of subjects not too large, we propose to use a highly adaptive lasso (HAL) estimator. This approach – while computationally intensive – has impressive performance and can readily be compared to the performance of more traditional lasso methods.

V. Environment

NC State provides the ideal environment in which to conduct this research. This project builds on research by Dr. Hoppin which leverages the expertise and experience of many members of the NC State Center for Human Health and the Environment (CHHE). CHHE, an NIEHS funded P30 center, is a multi-disciplinary environmental health science center which includes faculty from NC State, ECU, and North Carolina Central University. The CHHE Community Engagement Core has collaborated on the GenX Exposure Study and its leadership (Ms. May) will be a key contributor to this project. With graduate programs and training grants in Toxicology and Bioinformatics, NC State provides opportunities to engage students throughout this project.

VI. Integration with Overall Center

This BMR project is well integrated into the overall SRP Center (Figure 4). Through our community-engaged framework we will be working with the same Partners as the Community Engagement Core to ensure that our target communities are well represented both in our study and in access to information about PFAS. All chemical analyses will be conducted in CAPTURE using state of the art mass spectrometry. Dr. David Reif, DMAC leader will be a co-investigator on this project; he will provide expertise on toxicokinetics while the DMAC will provide quantitative estimates of PFAS exposure measured by CAPTURE. RETCC trainees will be invited to participate in data collection and report back activities to round out their training in environmental health science. The other projects complement this project and provide information of interest to the study community: namely, what are the health effects (immunotox)?, what are the implications for fish and wildlife (Bioaccumulation)?, and how can we reduce exposure (Water Treatment and Remediation). Results of these projects will be part of Center report back activities.

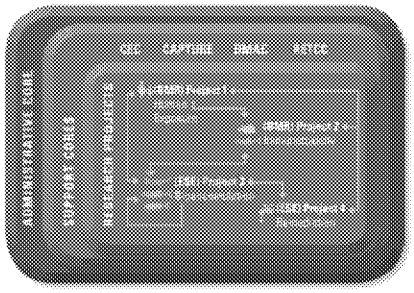

Figure 4: Center Integration

(BMR) Project #1: Human Exposure Center Integration Summary			
	Center Entity	**Contribution FROM**	**Contribution TO**
Core	Administrative	Provide administrative and fiscal management support; facilitate interaction, integration, and translation of research results	Provide critical data to facilitate progress, integration, and translation of research projects
	CEC	Participate in community meetings; help develop communications materials to anchor community- based decisions	Facilitate researcher engagement and multidirectional communication with affected communities
	DMAC	Provide data to explore new methods for mixture evaluation	Analyze raw MS data from untargeted analysis of human samples; final quality assurance check of all analyses
	RETCC	Provide training in human subjects research; provide opportunities to report back information; contextualize data	Allow trainee participation in research and report back to affected communities
	CAPTURE	Provide human samples for targeted and untargeted analysis	Provide identity and levels of PFASs in human samples
Research Project	Immunotoxicity	Provide guidance on prioritizing PFASs of greatest significance to human populations; provide data to assist in modeling "real world" mixtures	Provide information on potential immunotoxic outcomes of PFAS exposure
	Bioaccumulation		Provide information on bioaccumulation of PFASs and insight into potential routes of exposure for sharing with communities
	Water Treatment & Remediation		Provide information on drinking water exposure levels and efficacy of treatment options for affected populations

VII. Timeline